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Suppression of experimental allergic encephalomyelitis in rats by 50-nT, 7-Hz amplitude-modulated nocturnal magnetic fields depends on when after inoculation the fields are applied

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Abstract

Female Lewis rats (n = 88) were inoculated with an emulsion of spinal cord and complete Freund's adjuvant. They were then exposed in 11 separate blocks of experiments over a year period for approximately 6 min every hour between midnight and 08:00 h during post-inoculation nights 1–7, 8–16, 1–16, or 9 and 10 to 50-nT, 7-Hz, amplitude-modulated magnetic fields or to sham field (control) conditions. Compared to the control rats those exposed to the magnetic fields for nights 1–7 and nights 9–10 displayed more severe clinical symptoms while those exposed for nights 1–16 or 8–16 showed less severe symptoms. There was a strong correlation between the severity of the clinical symptoms in the control groups and the global geomagnetic activity 9 and 10 days after inoculation. These results suggest that the immunosuppressive effects of weak nocturnal magnetic fields may depend upon when they are applied during various stages in the development of a disease.
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Experimental simulation of the very low intensities and complex temporal patterns of geomagnetic activity have been shown to produce the same effects as those reported in correlational studies [11,14–16]. Cook and Persinger [4] showed that Lewis rats displayed less severe overt symptoms of experimental allergic encephalomyelitis or EAE [21] and fewer intracerebral infiltrations of lymphocytes when exposed between midnight and 08:00 h local time for 6 min per hour to a 7-Hz magnetic field whose amplitude was modulated about once every 30 s between 0 and 50 nT. The effect size of this treatment was similar in magnitude to effects of injecting syngenic adult neural stem cells in mice [17].

On the other hand, rats that had been exposed to these 7-Hz fields with stronger intensities (500 nT) or to 40-Hz fields presented with maximum intensities of either 50 or 500 nT did not display this suppression of symptoms or the diminished infiltration of mononuclear cells into the brain. The values of 7-Hz, 50-nT, 6-min durations, and amplitude plateaus of either 7 or 14 s had been selected to simulate some of the characteristics of most sudden storm commencements within the geomagnetic field and to be commensurate with the concept of temporal sensing [8].

In the present study, we hypothesized that nightly exposures to the same 7-Hz, 50-nT amplitude magnetic fields that produced suppression of clinical symptoms when the rats were exposed nightly from inoculation to termination [4,5,15] might exacerbate the symptoms if the full course of the treatment was not administered. In other experiments [12] Lewis rats in which limbic seizures were induced by lithium and pilocarpine immediately following inoculation with spinal cord emulsion showed exacerbation of the clinical symptoms of EAE compared to the non-seized reference group while those seized one week after the inoculation did not display any overt symptoms.

We decided to expose the inoculated rats to the sham field conditions or to the magnetic fields during the first half (nights 1–7), second half (nights 8–16), or the entire (nights 1–16)

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developmental stage of this process. The results of our first eight blocks had shown that the global geomagnetic activity (aa values) on the 9th and 10th day after inoculation was strongly ($r = .80$) correlated with the severity of the symptoms in the control rats. This observation was important potentially because there is a change in this cell-mediated immune response between 9 and 10 days after inoculation. Circulating levels of corticosterone [3], tumour necrosis factor-alpha, interferon gamma [6], and beta-endorphin, [18] as well as tissue levels of peroxynitrate, the most reactive oxygen species [3], and inducible nitric oxide synthase [3] increase at this time. Consequently, we exposed additional groups to the experimentally induced nocturnal magnetic fields only during the 9th and 10th nights after inoculation.

Eighty-eight female Lewis rats (aged 16–20 weeks of age), obtained from Charles River, Inc. (Montreal, Quebec), were employed as subjects in 11 separate blocks of experiments between February 2001 and February 2002. All rats were injected in both hindfoot pads with fresh female Lewis rat spinal cord emulsified in Freud's complete adjuvant (40 mg/kg; 0.1 cm^3 injection/footpad). Immediately following inoculation, the rats were weighed and placed sequentially in each of four separate cages until there were two per cage. Each of two plastic cages (32 cm × 36 cm × 45 cm) was surrounded along its major axis by a coil (30 Ω) of wire (AWG 20). Each of the two cages fit tightly within the each of the two coils whose proximal sides were separate by 5 cm. They were connected to a custom built output for a Model EU 81A Heath–Schlumberger function generator. Two other identical cages were maintained 30 cm below the two coils and housed the control groups. The rats remained in these cages, whose wood shavings (bedding) were refreshed once every 4 days, for the entire study. Food (daily on the bedding) and water were ad libitum.

When the field was activated by the Commodore computer, the intensity increased in sequential steps seven times during the first 3 min and 20 s and seven times during the last 1 min and 42 s. The duration of each plateau was 14 s during the first cluster and 7 s during the last cluster. The time required to change to each increment of intensity was 2 μs; the pattern has been published previously [4,5]. The total exposure time was 5 min and 50 s every hour from 00:00 h to 07:06 h. Room temperature was automatically controlled within one degree of 20 °C. The light:dark cycle was 12 h:12 h with the onset time of 08:00 h. The ambient level of light during the day cycle ranged between 700 and 800 lx in all four cages.

Blocks consisted of four rats exposed within the two coils and four rats housed within the control area below the coils. The long axes of the four cages were oriented in a N–S direction. Background characteristics of the geomagnetic field and procedures for verifying the presence and intensity of the experimental magnetic field (50 nT ± 10%) have described elsewhere [4,5]. The strength of the field generated within each Helmholtz coil was relatively uniform throughout the major area within which the rats were exposed. However, within each coil there was small gradient across the minor axis of about 8–10% so that the intensity on the side adjacent to the other coil was slightly less, e.g., a drop from 50 to 45 nT of the time-varying field. If any stray fields were present within the lower (control) cages the values were less than 10 nT (the limits of the sensor). The potential penetration of intermittent magnetic fields from the adjacent room through the walls into the experimental cages was eliminated by prohibiting the use of electronic equipment within these areas.

The rats housed in coils were exposed to the magnetic fields for nights 1–16 ($n = 8$), 1–7 ($n = 12$), 8–16 ($n = 12$), and 9–10 ($n = 12$, but one animal died during treatment); a total of 44 rats were exposed during these periods to the control (<10 nT) areas. On post-inoculation days 13–16, all the rats were weighed, examined, and rated on a scale of 0–5 [8] for clinical symptoms (0 = normal, 1 = tail atonia, 2 = ataxia, 3 = partial hindlimb paresis, 4 = complete hindlimb paresis and incontinence, 5 = complete paresis and death). The symptoms were qualitatively obvious and apparent even to naive observers; the ratings showed an inter-rater reliability greater than $\rho = .90$. On day 16 all rats were weighed and decapitated. Wet weights of thymuses and spleens were measured to the nearest 1 mg. There were no statistically significant differences [$F(4,63) = 1.30$, $p > .05$] between the groups for the initial body weights; the grand mean and standard deviation were 228 and 17 g, respectively.

The brains were fixed in an ethanol–formalin–acetic acid solution and processed [13]. Coronal, 10 μm sections were taken at equal distances between the area postrema and anterior commissure and stained with toluidine blue 0. The numbers of mast cells within the posterior (level of the medial geniculate), middle (level of the habenula) and anterior (level of the anterioventral nuclei) of the thalamus as defined previously [13] were counted at 400× magnification. Mast cells were counted because of their distinct morphology and because their numbers increase around intraparenchymal blood vessels 7 days after inoculation but decrease by day 10 [18]. We have found that decreased numbers of mast cells (even occasionally in the thalamus of the weanling Wistar rat) are associated with infiltrations of mononuclear cells that exhibit a spatial (concentration) gradient around local vasculature.

The numbers of foci (defined as conspicuous perivascular clusters of mononuclear cells not found in normal parenchyma) for each structure between the area postrema and the rostral Islands of Calleja were counted in a total of 13 equally spaced slides. The mean of the numbers of foci within a structure, which involved between 1 and 4 slides, was employed as datum. The means for the EAE scores for each of the 3 days examined as well as the relative weight loss ((final weight-initial weight)/initial weight) were calculated. All statistics analyses involved SPSS software. Post hoc tests were Tukey's ($p < .05$). To detect the possible influence of geomagnetic activity, daily global A values (average of the minimum and maximum) were obtained from the Geomag-

Table 1
Means (*M*) and standard errors of the mean (S.E.M.) for the severity of clinical scores, numbers of foci containing lymphocytic aggregates within the brain, predictions of clinical severity by the maximum linear combination of foci in brain structures within the right (RH) and left hemisphere (LH), numbers of mast cells in the thalamus (3, 10 μm sections), changes in body weight, and wet spleen and thymus weights for rats exposed to various treatments

	Nights of exposure to 7-Hz amplitude-modulated 50-nT field									
	Control		1–16		1–7		8–16		9–10	
	M	S.E.M.	*M*	S.E.M.	*M*	S.E.M.	*M*	S.E.M.	*M*	S.E.M.
Clinical signs										
Maximum score	1.6[b]	.3	.6[a]	.2	2.6[c]	.5	.5	.6	2.0[c]	.6
Total score	.8	.2	.4[a]	.2	1.5[b]	.4	.4[a]	.1	1.6[b]	.4
Predicted (RH)	1.4[b]	.2	.6[a]	.1	2.7[c]	.5	.7[a]	.1	1.7	.6
Predicted (LH)	1.5[b]	.3	.7[a]	.3	2.3[c]	.4	.8	.2	2.3	.6
Brain foci										
Numbers (all)	72	10	42	16	86	16	43	11	83	14
Mast cells (total numbers)										
Thalamus	68	4[a]	80[b]	8	52[c]	3	68[a]	4	65	8
Weights										
Change (body)	−3%[a]	1	+2%[b]	2	−4%[a]	1	0%[b]	1	0%[b]	2
Thymus (mg)	231	12	288[a]	12	202[b]	18	228	22	261	22
Spleen (mg)	452[a]	7	504[b]	16	449	12	437[a]	9	485	35

a vs. b: $p < .05$; b vs. c: $p < .05$.

netic Indices Bulletin (Boulder, CO) and converted to provisional global aa [10] values (in nanoTesla) for the northern hemisphere.

The means and standard errors of the means (S.E.M.) for several measures for the groups exposed to the control condition or to the experimental magnetic fields for 16 nights, nights 1–7, nights 8–16, or only on nights 9 and 10 after the inoculation are shown in Table 1. These measures were: maximum severity of clinical signs (to emphasize the greatest single daily severity), the average of the daily clinical signs for 4 days (post-inoculation days 13–16), the total numbers of foci of lymphocytes, the predicted maximum score for clinical severity based upon the linear combination of numbers of foci for the first eight structures that entered the multiple regression equations for the left and right side of the brain separately, the numbers of mast cells in the thalamus, and the percentage changes in body weights. There were statistically significant ($p < .05$) differences (all *F*s between 2.71 and 4.95, all d.f.s = 4,82) between the groups for the total clinical scores, the maximum clinical scores, the equations for the maximum score based upon the numbers of foci within the left or right side of the brain, the numbers of mast cells in the thalamus, and the percentage loss of body weight. The treatment explained between 17 and 23% in the variance for these measures.

Compared to the control group the rats that had been exposed to the magnetic fields for 16 successive nights or from nights 8 to 16 after inoculation displayed less intense maximum clinical scores than the groups exposed during the first seven nights after inoculation or during the 9th and 10th nights after inoculation. The significant differences between the groups for wet thymus weights ($F = 2.76$, $\eta^2 = 15\%$) and spleen weights ($F = 3.19$) were due primarily to the heavier weights for both tissues in the group exposed for 16 nights compared to the control group or the ones exposed on nights 8–16.

The differences between groups for the total numbers of foci within the brains were not significant statistically. When the group means for these values were correlated for the five groups (d.f. = 5), the correlations (r and ρ, respectively; values > absolute value of .8, $p < .05$) for the various pairs of variables were very strong. The combinations were: maximum clinical signs and numbers of foci (.98, .90), maximum clinical signs and numbers of mast cells in the thalamus (−.81, −.90), numbers of foci and numbers of mast cells in the thalamus (−.81; −.90) thalamus.

According to step-wise multiple regression the first eight structures within the right hemisphere whose numbers of foci were most strongly correlated with maximum clinical signs [multiple $r = .88$, $F(8,58) = 25.23$, $p < .001$] were the commissure of the superior colliculus, the posterior part of the anterior hypothalamic area, the external cortices of the inferior colliculus, the subbrachial nucleus, the bed nucleus of the stria terminalis (intraamygdaloid division), the longitudinal fasciculus of the pons, the anterior medial preoptic area, and the reuniens nucleus of the thalamus.

For the left hemisphere the top eight structures [multiple $r = .86$, $F(7,60) = 25.43$, $p < .001$] were the paraventricular thalamic nucleus, the external cortex of the inferior colliculus, oral part of the pontine nucleus, posterior part of the anterior hypothalamic area, the optic chiasm, CA3 of the hippocampus and the thalamic reticular nucleus. For all structures the partial slopes were positive. As shown in Table 1, the pattern of severity of the predicted clinical severity based upon either the left or right brain structures with lymphocytic infiltrations was similar to the observed clinical scores.

The grand mean and standard deviation for the estimated aa values for the 11 different 16-day series were 22.5 nT

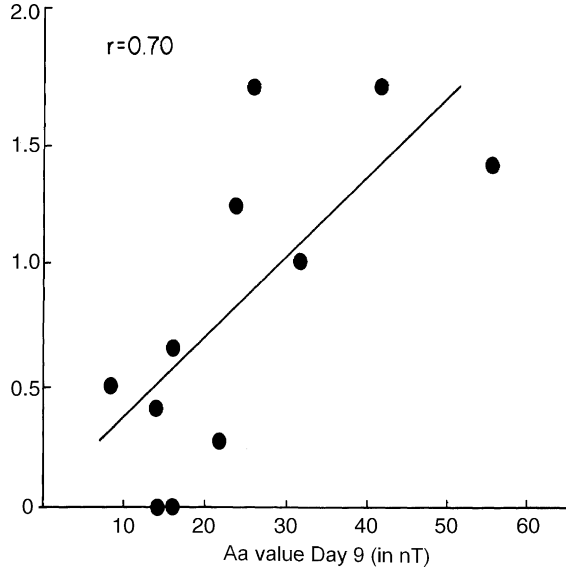

Fig. 1. Mean scores for clinical symptoms as a function of the geomagnetic activity (in nanoTesla) during the ninth day after inoculation for EAE in rats exposed to control condition.

(range 12–37 nT) and 6.8 nT. For the control rats, the most significant correlations occurred between the severity of the clinical symptoms and the amount of geomagnetic activity during days 9 and 10 after the inoculation. The rats exposed to any of the experimental magnetic fields did not display any statistically significant correlations between the daily geomagnetic values and their final clinical scores.

Fig. 1 shows the scattergram (r = .70, p < .01) between the clinical scores and the geomagnetic activity on day 9 for the groups of rats exposed to the control condition. The correlation between final clinical scores and the activity on post-inoculation day 10 was a more linear r = .82 (p < .01); the slope was similar to that shown in Fig. 1. To discern if there was a critical day for this correlation between clinical severity for the control group and geomagnetic activity, partial correlation analyses were completed between the severity of the clinical scores and for geomagnetic activity on day 9 or 10 post-inoculation with the variance of the other day first removed.

For the activity on day 10, the partial correlation was .65 after the shared variance with day 9 was removed; when the reversed was analyzed the significant correlation with day 9 was eliminated. Similarly the correlation (r = .68) between the average geomagnetic activity for the entire period of the experiment and clinical scores was eliminated when geomagnetic activity on day 10 was held constant. Holding the former constant did not affect the strength of the association for day 10 (r = .70).

The results of the present study replicated previous research that 6-min exposures once per hour during the night phase to 50-nT, 7-Hz amplitude-modulated magnetic fields reduced the severity of the clinical symptoms of EAE. The results also showed that exposure for only the first seven nights may encourage a rebound that exacerbates the symptoms when they later develop. We found similar patterns of facilitation or suppression of clinical symptoms when electrical seizures were induced by a single systemic injection of lithium (3 mEq./kg) and pilocarpine (30 mg/kg) immediately or 1 week after inoculation [12].

Clearly the most significant result was the exacerbation of the symptoms when rats were exposed on only post-inoculation days 9 and 10. Increased severity of the symptoms was also noted in the control groups over the 11 blocks of experiments if there had been greater global geomagnetic activity during this post-injection period. The range of the severity of the symptoms in the control group was similar to those produced by the experimental fields.

There are three implications from these similarities. First, exposure to only eight 6-min periods during two nights, or approximately 3% of the total time is required to produce the enhancement of symptoms. Secondly the slope of the relationship between the symptoms and the ambient geomagnetic activity might suggest a threshold of around 20–30 nT, which is similar to the values obtained for other behaviors such as accuracy for homing in pigeons [20] and limbic seizures in rats [2]. Third, there may be very specific temporal windows during the development of a disease where either the geomagnetic activity or experimental magnetic fields maximum affect its outcome. These results are consistent with Sandyk's hypothesis [19] that the spontaneous variations in the activity of multiple sclerosis could be triggered in part by the daily, circadian, seasonal, or annual changes in the intensity of the geomagnetic field that affects the immunomodulatory influence of the pineal organ upon the body. The involvement of pineal melatonin [9,19], an endogenous compound whose rate of synthesis peaks during the night and whose levels are negatively correlated with geomagnetic activity [1] and are sometimes but not always suppressed by experimental magnetic fields [7], must still be explored.

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